UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

February 27, 2014

MEMORANDUM

Subject: Efficacy Review for Klorsept; EPA Reg. # 71847-6; DB Barcode: D415823.

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Applicant: Medentech Ltd c/o Clearon Corp.
95 MacCorkle Avenue S.W.
South Charleston, WV 25303

Formulation from the Label:

Active Ingredients	% by wt.
Sodium dichloro-s-triazinetrione	48.21 %
Other Ingredients	51.79 %
Total	100.00 %

I. BACKGROUND

The product, Klorsept (EPA Reg. No. 71847-6), is an EPA-approved disinfectant with sanitizing effects, for use on hard, non-porous surfaces in institutional, household, commercial, and hospital or medical environments. The applicant requested an amendment to the registration of this product to add disinfectant claims for pathogens and marketing language. Studies were conducted at MicroBioTest, Inc., located at 105B Carpenter Drive in Sterling, VA 20164.

This data package identified as D415823 contained a letter from the applicant's representative to EPA (dated September 30, 2013), EPA Form 8570-35 (Data Matrix), five studies (MRID Nos. 492306-01 through 492306-05), Statements of No Data Confidentiality for all five studies, and the proposed label (dated 01/10/2014).

II. USE DIRECTIONS

The product is designed to be used for disinfecting hard, non-porous surfaces such as door handles, clean-up carts, light switches, sinks, tubs, tiles, toilets, shower doors, floors, dressing or linen carts, hampers, diaper pails, toilet seats, bed pans, plastic mattress covers, lockers... Directions on the proposed label provided the following information regarding use of the product as a:

DISINFECTION/VIRUCIDAL DIRECTIONS:
[Prepare a 958ppm solution; refer to dilution chart]. Apply use solution to visible cleaned, hard, non-porous, inanimate surfaces with brush, spray device, sponge, cloth, or mop to wet all surfaces thoroughly. Allow to remain wet for 10 minutes, then remove product by wiping with brush, sponge, or cloth.
For sprayer applications use a coarse spray device. Spray 6-8 inches from surface, and rub with brush, sponge, wipe or cloth. Do not breathe spray mist.

SANITIZER FOR FOOD AND BEVERAGE PROCESSING AND FOOD HANDLING OPERATIONS:
This product is recommended for sanitizing all types of hard, non-porous equipment and utensils used in food processing and canning plants, bottling plants, breweries, fish processing plants, meat and poultry processing plants, milk handling and processing plants, stores, restaurant and institutional dining establishments. Use a 100 ppm available chlorine solution [(refer to Dilution Chart)] to sanitize previously cleaned processing and packaging equipment. [Add 1 tablet to 1 quart {optional statement to be used only for 334 mg tablet.}] Allow at least a one minute contact time before draining. Allow adequate draining before contact with beverages.]

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then

treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant at LCL must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Virucides - Use of a Surrogate Virus: For certain viruses, there are no *in vitro* systems or *in vivo* animal models (except for humans and chimpanzees). The Agency permits the testing of surrogate viruses in these cases, for example, Bovine viral diarrhea virus as a surrogate for human Hepatitis C virus, Duck Hepatitis B virus as a surrogate for Human Hepatitis B virus, and Feline calicivirus as a surrogate for Norwalk virus.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products) may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum.

IV. BRIEF DESCRIPTION OF THE DATA

Note: The product were tested at concentrations presented in the following table:

Product Lot(s)	Tested Concentration(s)
02213	937 ppm, 140 ppm, 75 ppm, 100 ppm, 125 ppm
02513	937 ppm, 140 ppm, 75 ppm, 100 ppm, 125 ppm
13DD241	75 ppm, 100 ppm, 125 ppm
C495	1 = 988 ppm, 2 = 1975 ppm, 3 = 2469 ppm
C638	1 = 990 ppm, 2 = 1980 ppm, 3 = 2477 ppm
7055	985 ppm
6555	972 ppm

1. MRID 492306-01 "AOAC Available Chlorine Germicidal Equivalent Test, Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Salmonella typhi* (ATCC 6539)" for Klorsept (Agrisept® MC tabs), by M. Hamid Bashir. Study conducted at MicroBioTest. Study completion date – July 29, 2013. Project Number 642-129.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Salmonella typhi* (ATCC 6539) grown in nutrient broth. Three lots (02213, 02513, and 13DD241) of the product, Klorsept (Agrisept® MC tabs), were tested according to MicroBioTest Protocol No. 642.1.06.19.13 (copy provided). The product was diluted to 75, 100, and 125ppm using 400ppm±2.9% AOAC Hard Water. Sodium hypochlorite (NaOCl) was used as the data control standard at 100 ppm (titrated at 99.4 ppm). Letheen Broth with 0.2% Sodium Thiosulfate was used as neutralizer; and Tryptic Soy Agar was used as agar plate medium. A 0.05 ml aliquot of the test culture was added to each (10 ml) of the test substance and control NaOCl solutions at 20±1°C. One minute after addition of the test organism, one loopfull of each medicated culture was transferred to the subculture medium using a flamed 4 mm loop. Each tube was then challenged with additional 0.05 ml aliquot of the test culture 30 seconds after subculturing. This process was repeated for a total of 10 subcultures (at these contact times: 1, 2.5, 4, 5.5, 7, 8.5, 10, 11.5, 13, 14.5 minutes) for each lot and control. The neutralized subcultures were incubated for 48±2 hours at 37±2°C in ambient air, and examined for the presence or absence of visible growth. Representative neutralized subcultures showing growth were subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism. Controls included neutralization, viability control, purity, and sterility.

2. MRID 492306-02 "Virucidal Hard-Surface Efficacy Test; Utilizing Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus", by Zheng Chen. Study conducted at MicroBioTest. Study completion date – July 01, 2013. Project Number 642-127.

This study was conducted against the strain Grimaud of duck Hepatitis B virus (BHBV) (obtained from Hepadnavirus Testing Inc., Palo Alto, CA), using primary duck hepatocytes (PDH) as the host system. Two lots (02213 and 02513) of the product, Klorsept (Agrisept® MC tabs), were tested according to MicroBioTest Protocol No. 642.1.06.12.13 (copy provided). The product was diluted to 937 ppm using 400ppm±2.9% AOAC Hard Water. The stock virus culture contained whole duck serum (100% duck serum) as organic load. Films of virus were prepared at staggered intervals by spreading 400 µL of virus uniformly over 2x2 inch area of glass carrier and dried for 30 minutes. For each batch of test substance assayed, two dried virus films were individually exposed to 2 mL of test substance and held covered for 10 minutes at room temperature (21.0°C) under 45.3-46.2% relative humidity. At the end of the exposure time, 2 ml of neutralizer (Complete L-15 with 0.01% $Na_2S_2O_3$ and 10% fetal bovine) the dried films were scraped with a cell scraper. The virus-test substance-neutralizer mixtures were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. Test medium used to maintain the cell cultures was Complete Liebovitz L-15. PDH cells in multiwell culture dishes were inoculated in quadruplicate with the dilutions from the test and control groups and were incubated at 36±2°C in a humidified atmosphere of 5±1% CO_2. The cultures were scored periodically for 10-14 days for the absence or presence of CPE, cytotoxicity, and for viability. In addition, the plates were assayed by immunofluorescence assay. Host cells were fixed with tissue culture grade alcohol, stained and read for infectivity, and enumerated. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The titer of the dried virus control was **4.85 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was **3.75 $\log_{10}$** for all batches.

3. MRID 492306-03 "Virucidal Hard-Surface Efficacy Test against Rhinovirus Type 14 (ATCC VR-284)", for Klorsept; by S Steve Zhou. Study conducted at MicroBioTest. Study completion date – February 11, 2013. Project Number 642-123.

This study was conducted against Rhinovirus Type 14 (ATCC VR-284), using H1-HeLa cells (ATCC CCL-1958) as the host system. Two lots (C495 and C638) of the product, Klorsept (Agrisept® MC tabs), were tested according to MicroBioTest Protocol No. 642.1.01.18.13 (copy provided). The product was diluted to concentrations ranging from 988 ppm to 2477 ppm using 400ppm±2.9% AOAC Hard Water.. The stock virus culture contained a 5% organic soil load. Films of virus were prepared at staggered intervals by spreading 400 µL of virus uniformly over the bottoms of six 100 x 15 mm sterile glass petri dishes and dried at 20.0°C for 30 minutes at 9.3-9.9% relative humidity. For each batch of test substance assayed, one dried virus film was exposed to 2 mL of test substance and held covered for 1 0 minutes at room temperature (20.0°C) under 8.9-9.3% RH. At the end of the exposure time, 2 ml of neutralizer (RPMI with 0.01% $Na_2S_2O_3$ and 10% fetal bovine) and the dried films were scraped with a cell scraper. The virus-test substance-neutralizer mixtures were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. Test medium used to maintain the cell cultures was RPMI+2% Fetal Bovine Serum. H1-HeLa cells in multiwell culture dishes were inoculated in 8 replicates with the dilutions from the test and control groups and were incubated at 33±2°C in a humidified atmosphere of 5±1% CO_2. The cultures were scored periodically for 6-9 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The titer of the dried virus control was **6.78 log_{10}**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was **3.38 log_{10}** for each one of 3 concentrations per batch and for both batches.

4. MRID 492317-04 "Virucidal Hard-Surface Efficacy Test against Respiratory syncytial virus (ATCC VR-26, Strain Long)", for Klorsept (Agrisept® MC tabs); by Michael Parker. Study conducted at MicroBioTest. Study completion date – July 11, 2013. Project Number 642-128.

This study was conducted against Respiratory syncytial virus, Strain Long (ATCC VR-26), using HeLa cells (ATCC CCL-2) as the host system. Two lots (02213 and 02513) of the product, Klorsept (Agrisept® MC tabs), were tested according to MicroBioTest Protocol No. 642.2.06.12.13 (copy provided). The product was diluted to 140 ppm using 400ppm±2.9% AOAC Hard Water. The stock virus culture contained a 5% organic soil load (fetal bovine serum). Films of virus were prepared at staggered intervals by spreading 400 µL of virus uniformly over the bottoms of six 100 x 15 mm sterile glass petri dishes and dried at 20.0°C for 30 minutes. For each batch of test substance assayed, one dried virus film was exposed to 2 mL of test substance and held covered for 10 minutes at room temperature (20.0°C) under 44.8-45.6% RH. At the end of the exposure time, 2 ml of neutralizer (DMEM with 0.01% $Na_2S_2O_3$ and 10% fetal bovine) and the dried films were scraped with a cell scraper. The virus-test substance-neutralizer mixtures were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. Test medium used to maintain the cell cultures was DMEM+2% Fetal Bovine Serum. HeLa cells in multiwell culture dishes were inoculated in 8 replicates with the dilutions from the test and control groups and were incubated at 36±2°C in a humidified atmosphere of 5±1% CO_2. The cultures were scored periodically for 14-18 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The titer of the dried virus control was **6.40 log_{10}**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was **4.00 log_{10}** for both batches.

5. MRID 492317-04 "AOAC Fungicidal Effectiveness Test against *Microsporum canis* (ATCC 44457)", for Klorsept (Bru-Clean Tbc); by Travis R. Farley. Study conducted at MicroBioTest. Study completion date – June 26, 2008. Project Number BRU.1.07.10.07.

This study was conducted against *Microsporum canis* (ATCC 44457). Two lots (02213 and 02513) of the product, Klorsept (Bru-Clean Tbc), were tested according to MicroBioTest Protocol No. BRU.1.07.10.07 (copy provided). The product lot were diluted to 985 ppm and 972 ppm using one gallon of 400-410ppm AOAC Hard Water. No organic soil was added to the inoculum. Two (2) tubes of 5 ml per product lot were inoculated with 0.5 ml of a suspension of fungal inoculum. The tubes were swirled and allowed to remain for 10 minutes. Following exposure, a loop-full of conidia-test substance mixture from each tube was transferred into 10 mL of Potato Dextrose Broth containing 7% Polysorbate 80, 1% Lecithin, and 0.3% $Na_2S_2O_3$, neutralizing subculture media. Thirty minutes after, a loop was transferred to a secondary growth medium (Potato Dextrose Broth). All subcultures were incubated for at least 10 days at 25-30°C. Following incubation, the subcultures were examined for growth. Controls included those for purity, sterility, viability, fungistasis, and initial counts. The average conidia forming units of inoculum was **7.8×10^5/ml**.

V. RESULTS

MRID 492306-01	***Staphylococcus aureus***										***Salmonella typhi***									
Subcultures	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
100ppm Available Chlorine - NaOCl Control Solution																				
100 ppm	0	0	0	0	+	+	+	+	+	+	0	0	0	0	0	+	+	+	+	+
Lot # 02213																				
75 ppm	0	0	0	+	+	+	+	+	+	+	0	0	0	0	+	+	+	+	+	+
100 ppm	0	0	0	0	+	+	+	+	+	+	0	0	0	0	0	+	+	+	+	+
125 ppm	0	0	0	0	0	+	+	+	+	+	0	0	0	0	0	0	+	+	+	+
Lot # 02513																				
75 ppm	0	0	0	+	+	+	+	+	+	+	0	0	0	0	+	+	+	+	+	+
100 ppm	0	0	0	0	+	+	+	+	+	+	0	0	0	0	0	+	+	+	+	+
125 ppm	0	0	0	0	0	+	+	+	+	+	0	0	0	0	0	0	+	+	+	+
Lot # 13DD241																				
75 ppm	0	0	0	+	+	+	+	+	+	+	0	0	0	0	+	+	+	+	+	+
100 ppm	0	0	0	0	+	+	+	+	+	+	0	0	0	0	0	+	+	+	+	+
125 ppm	0	0	0	0	0	+	+	+	+	+	0	0	0	0	0	0	+	+	+	+

MRID	**Organism**	**No. Exhibiting Growth/ Total No. Tested**		**Inoculum Count (CFU/ml)**
		Lot 7055	**Lot 6555**	
492306-05	*Microsporum canis*	0/2	0/2	7.8×10^5

MRID Number	Organism	Results @ 10 Minutes Contact Time			Dried Virus Control (log_{10} $TCID_{50}$/0.4mL)
		Description	Lot # 02213	Lot # 02513	
492306-02	Duck Hepatitis B virus	10^{-2} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation	4.85
		Log_{10} $TCID_{50}$/0.4 mL	≤1.10	≤1.10	
		Log Reduction	≥3.75	≥3.75	
492306-04	Respiratory syncytial virus, Strain Long	10^{-2} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation	6.40
		Log_{10} $TCID_{50}$/0.4 mL	≤2.40	≤2.40	
		Log Reduction	≥4.00	≥4.00	
			Lot # C495	**Lot # C638**	
492306-03	Rhinovirus Type 14	10^{-2} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation	6.78
		Log_{10} $TCID_{50}$/0.4 mL	1 = ≤3.40 2 = ≤3.40 3 = ≤3.40	1 = ≤3.40 2 = ≤3.40 3 = ≤3.40	
		Log Reduction	1 = ≥3.38 2 = ≥3.38 3 = ≥3.38	1 = ≥3.38 2 = ≥3.38 3 = ≥3.38	

VI. CONCLUSION

1. The submitted efficacy data, MRID 492306-01, support the use of the product, Klorsept (EPA Reg. No. 71847-6), as a food contact rinse sanitizer against *Staphylococcus aureus* (ATCC 6538) and *Salmonella typhi* (ATCC 6539), on pre-cleaned hard nonporous surfaces, when diluted to 100 ppm available chlorine with 400 ppm hard water, at room temperature for a contact time of at least a one minute.

- Colony forming units per ml in the initial suspensions population was not reported for the test.
- NaOCl control solutions were not tested at 200, 100, and 50 ppm (µg/mL) available Chlorine.

2. The submitted efficacy data, MRID 492306-02, support the use of the product Klorsept (EPA Reg. No. 71847-6), as a disinfectant with virucidal activity against Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus, on hard, nonporous surfaces, when diluted to 937 ppm available chlorine with 400 ppm hard water, in the presence of a 5% organic soil load at room temperature for a contact time of 10 minutes.

3. The submitted efficacy data, MRID 492306-03, support the use of the product Klorsept (EPA Reg. No. 71847-6), as a disinfectant with virucidal activity against Rhinovirus Type 14 (ATCC VR-284), on hard, nonporous surfaces, when diluted to 990 ppm available chlorine with 400 ppm hard water, in the presence of a 5% organic soil load at room temperature for a contact time of 10 minutes:

4. The submitted efficacy data, MRID 492306-04, support the use of the product Klorsept (EPA Reg. No. 71847-6), as a disinfectant with virucidal activity against Respiratory syncytial virus, Strain Long (ATCC VR-26), on hard, nonporous surfaces, when diluted to 140 ppm available chlorine with 400 ppm hard water, in the presence of a 5% organic soil load at room temperature for a contact time of 10 minutes:

5. The submitted efficacy data, MRID 492306-05, **do not support** the use of the product, Klorsept (EPA Reg. No. 71847-6), as a disinfectant with fungicidal activity against *Microsporum canis* (ATCC 44457), on pre-cleaned hard nonporous surfaces, when diluted to 985 ppm available chlorine with 400 ppm hard water, at room temperature for a contact time of 10 minutes. Only 2 tubes out of 10 were tested.

VI. LABEL

1. General Disinfection claim at 479 ppm available chlorine is misleading. The must list organisms that product is effective against at that concentration or remove the claim. See page 5 of the proposed label just before "*Number 2 alternate disinfection application*".

2. The proposed label claims **are acceptable** regarding the use of the product, Klorsept (EPA Reg. No. 71847-6), as a food contact rinse against , on hard non-porous surfaces, when diluted to 100 ppm available chlorine, for at least 1 minute contact time. These claims **are supported** by the applicant's data.

3. The proposed label claims **are acceptable** regarding the use of the product, Klorsept (EPA Reg. No. 71847-6), as a disinfectant for use on hard, non-porous surfaces against Duck Hepatitis B Virus or Human Hepatitis B Virus, when diluted to 958 ppm available chlorine, in the presence of 5% organic soil, at room temperature for 10 minutes contact time. These claims **are supported** by the applicant's data.

4. The proposed label claims **are acceptable** regarding the use of the product, Klorsept (EPA Reg. No. 71847-6), as a disinfectant for use on hard, non-porous surfaces against Respiratory syncytial virus, Strain Long (ATCC VR-26), when diluted to 479 ppm available chlorine, in the presence of 5% organic soil, at room temperature for 10 minutes contact time. These claims **are supported** by the applicant's data.

5. Claim for Rhinovirus Type 14 is under marketing statements without product effectiveness concentration a contact time. **The registrant must list effective concentration against Rhinovirus Type 14 as at least 990 ppm available chlorine for 10 minutes contact time**.

6. The proposed label claims **are not acceptable** regarding the use of the product, Klorsept (EPA Reg. No. 71847-6), as a disinfectant for use on hard, non-porous surfaces against *Microsporum canis* (ATCC 44457), when diluted to 958 ppm available chlorine, in the presence of 5% organic soil, at room temperature for 10 minutes contact time. **The registrant must remove all *Microsporum canis* (ATCC 44457) claims from the label**.

7. Rhinovirus Type 14 and Respiratory syncytial virus, Strain Long's reference numbers or ATCC numbers must be included in the data matrix.